SEVERINE N-OXIDE AND KORSEMINE FROM *Korolkowia sewerzowii*

K. Samikov, D. U. Abdullaeva,
R. Shakirov, and S. Yu. Yunusov

UDC 547.944/945

Korsevine and the new alkaloids severine N-oxide and korsemine have been isolated from the combined alkaloids of *Korolkowia sewerzowii* Regel. On the basis of the results of a study of the IR and NMR spectra of the alkaloids and also of a correlation with korseveriline and korsevine it has been established that base (I) is severine N-oxide and korsemine has the structure of korsevine 3β-D-glucopyranoside.

Continuing an investigation of the total alkaloids of *Korolkowia sewerzowii* Regal [1, 2] we have isolated korsevine [3] and new alkaloids: one with mp 255-257°C (I) and korsemine (II). Korsemine has also been isolated from the epigeal part of this plant collected in Katrantau (Kirghiz SSR) [2].

The IR spectrum of (I) shows absorption bands at (cm^{-1}) 3380 (OH), 2980-2855, 1455 ($-CH_3$; $-CH_2-$), 1727, 1255 ($C=O$, ester), 970, 954, and 935 ($N \to O$) [4]. The mass spectrum of (I) has the peaks of ions with m/e 98, 111 (100%), 112, 124, 149, 162, 166, 178, 412, 417, 430, 440, 444, 453, 454, 458, 471, 472, 473, 489 M^+, which are characteristic for the C-nor-D-homosteroid alkaloid korseveriline [1, 5].

The following signals are observed in the NMR spectrum of (I): three-proton singlets at 0.94 ppm ($19-CH_3$) and 1.97 ppm ($OCOCH_3$), a six-proton doublet at 0.84 ppm ($21-CH_3$, $27-CH_3$), and a multiplet at 5.07 ppm (H, $\underline{H}C-OCOCH_3$) from a proton geminal to an acetoxy group [6].

The presence in the IR spectrum of bands characteristic for N-oxides, the presence in the mass spectrum of strong peaks of the $(M-16)^+$ and $(M-17)^+$ ions, and the ready solubility of alkaloid (I) in water showed its N-oxide nature. The reduction of (I) with zinc in hydrochloric acid gave substances with mp 144-146°C (benzene) and 240-242°C (acetone), identical, respectively, with severine and korseverine (mixed melting points, IR spectra) [1, 5, 7]. The saponification of (I) formed a substance with mp 259-260°C, M^+ 447, identical with korseveriline N-oxide, which we have obtained by the oxidation of korseveriline with hydrogen peroxide.

The oxidation of severine [7] with hydrogen peroxide led to an N-oxide identical with the alkaloid (I). Consequently, compound (I) is severine N-oxide and has the structure (I).

Korsemine is a tertiary unsaturated base which in a solution weakly acidified with sulfuric acid instantaneously decolorizes a potassium permanganate solution; this indicates the presence of a double bond. The IR spectrum of the base shows absorption bands at (cm^{-1}) 3400 (OH), 3057, 1635 ($>C=C<$), 1710 (carbonyl in a six-membered ring), and 1100-1000 cm^{-1} (broad absorption band characteristic for glycoalkaloids) [2, 8].

With acetic anhydride in pyridine, korsemine forms tetraacetylkorsemine (III). In the IR spectrum of (III) the absorption band of hydroxy groups has disappeared. The NMR spectrum of (III) has singlets at (ppm) 2.24 (3 H, $N-CH_3$), 0.60 (3 H, $19-CH_3$), 1.58 (3 H, $18-CH_3$), 2.02 (6 H, $OCOCH_3$), 1.97 and 1.95 (6 H, $OCOCH_3$), and doublets at 0.70 (3 H, $21-CH_3$), and 0.95 (3 H, $27-CH_3$).

Thus, korsemine is a glycoalkaloid based on the heterocyclic skeleton of korsevine [3]. In actual fact, the hydrolysis of korsemine in hydrochloric acid in the presence of ethanol led to an amino alcohol with mp 168-170°C (methanol) having the composition $C_{28}H_{45}NO_2$, M^+ 427, identical with korsevine (mixed melting point, IR, NMR, and mass spectra) [3]. D-Glucose was detected by paper chromatography in the neutral fraction of the hydrolysate after

---

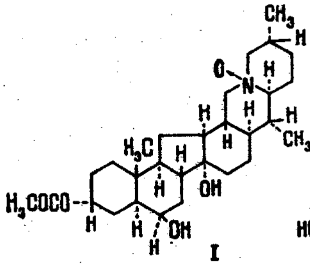
Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 823-826, November-December, 1979. Original article submitted July 11, 1979.

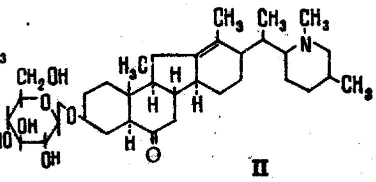
0009-3130/79/1506-0728$07.50

the separation of the aglycone. The presence of D-glucose in korsemine was also confirmed by gas—liquid chromatography [9]. The identity of the amino alcohol with korsevine was also confirmed by the preparation of their monoacetyl derivatives. The NMR spectrum of the acetylaglycone showed singlets at (ppm) 0.63 (3 H, 19-CH_3), 2.30 (3 H, N-CH_3), 1.55 (3 H, 18-CH_3), and 1.97 (3 H, $OCOCH_3$), doublets at 0.97 (3 H, 27-CH_3) and 0.71 (3 H, 21-CH_3), and a multiplet at 4.62 ppm (H,$\underline{H}C$—$OCOCH_3$) from a proton geminal to an acetoxy group.

The results of a determination of the difference in molecular rotations between korsemine and korsevine according to Klyne's rule [10] showed that in the korsemine molecule the D-glucose is attached to korsevine by a β-glycosidic bond.

On the basis of what has been said above, it may be assumed that korsemine has the structure of korsevine 3-β-D-glucopyranoside (II).


I


II

EXPERIMENTAL

For thin-layer chromatography (TLC) we used KSK silica gel (100 nm) and the chloroform—methanol (10:1) solvent system. The revealing agent was Dragendorf's solution. For column chromatography we used KSK silica gel (250 nm). IR spectra (KBr) were recorded on a UR-20 spectrometer, NMR spectra on a JNM-4H-100/100 MHz instrument ($CDCl_3$ with HMDS as internal standard; values given in the δ scale), and mass spectra on a MKh-1303 instrument fitted with a glass system for the direct introduction of the substance into the ion source.

Korsevine was obtained by a method described previously [3] from the petroleum ether fraction of the total alkaloids of the bulbs of Korolkowia sewerzowii [2], mp 168-170°C (methanol), M^+ 427, R_f 0.26.

Isolation of Severine N-Oxide. After the separation of the korseverine [1], the mother solution (0.5 g) was chromatographed on a column of silica gel with elution by ethyl acetate—petroleum ether—methanol (10:10:1). Fractions with a volume of 10-15 ml were collected. After concentration, combined fractions 16-19 yielded severine N-oxide with mp 255-257°C (methanol), $[\alpha]_D \pm 0$, $C_{29}H_{47}NO_5$, R_f 0.26.

Reduction of Severine N-Oxide. A solution of 0.1 g of severine N-oxide in 5 ml of 5% hydrochloric acid was treated with 0.5 g of granulated zinc and was left at room temperature for three days. Then the solution was separated off, made alkaline with ammonia, and extracted with chloroform. The residue after the distillation of the chloroform was treated with acetone. This led to the deposition of crystals with mp 240-242°C (acetone), identical with korseveriline. The mother solution after the isolation of korseveriline showed two spots with R_f 0.33 and 0.10 on TLC. The mixture was separated on a column of silica gel with elution by chloroform—methanol (10:1). Fractions with a volume of 8-10 ml were collected. The first 30 ml of eluate yielded a substance with mp 144-146°C (benzene) identical with severine, R_f 0.33, M^+ 473 (mass spectrometry). The subsequent eluates were combined and treated with acetone to give korseveriline with R_f 0.1.

Saponification of Severine N-Oxide. A solution of 20 mg of severine N-oxide in 1 ml of methanol was treated with 1.5 ml of a 5% methanolic solution of caustic potash. The reaction mixture was left at room temperature for 16 h, and then the methanol was evaporated off in vacuum. The residue was treated with water and extracted with chloroform. From methanol, crystals deposited with mp 259-260°C (methanol), R_f 0.07, identical with synthetic korseveriline N-oxide.

Oxidation of Severine. A solution of 0.1 g of severine in 4 ml of ethanol was treated with 0.5 ml of 30% hydrogen peroxide and the mixture was left at room temperature for three days. The ethanol was distilled off in vacuum and the residue was then diluted with water, made alkaline with ammonia, and extracted with chloroform. The latter was distilled off and the residue was dissolved in a mixture of benzene and methanol (10:1 and chromatographed on a column of silica gel. Elution was performed with benzene—methanol (10:1), 10-ml fractions being collected. The first 20 ml of eluates yielded severine (R_f 0.33) and the subsequent 40 ml, a base with mp 255-257°C (methanol), R_f 0.26, identical with severine N-oxide.

Oxidation of Korseveriline. A solution of 0.2 g of korseveriline in 7 ml of ethanol was treated with 1.2 ml of 30% hydrogen peroxide and oxidation was performed as in the case of severine. This gave korseveriline N-oxide with mp 259-260°C (methanol), R_f 0.07, identical with the saponified product of severine N-oxide.

Korsemine. The mother liquor from sevkorine [1, 2] was chromatographed on a column of silica gel using benzene—methanol (10:0.5, 10:1, and 4:1) as eluent. The benzene—methanol (4:1) eluates yielded korsemine, $[\alpha]_D - 46.9°$ (c 0.81; methanol), $C_{34}H_{55}NO_7$, R_f 0.10.

Tetraacetylkorsemine. A mixture of 0.05 g of korsemine in 1 ml of pyridine and 1 ml of acetic anhydride was kept at room temperature for three days. After the pyridine had been driven off, the residue was treated with a 5% solution of sulfuric acid. The acid solution was made alkaline with ammonia and the reaction product was extracted with chloroform. This gave the amorphous tetraacetylkorsemine with R_f 0.34.

IR spectrum, cm^{-1}: 1755, 1225 (C=O, ester), 1710 (C=O).

Hydrolysis of Korsemine. A mixture of 0.23 g of korsemine, 10 ml of ethanol, and 10 ml of 6% hydrochloric acid was boiled for 3 h. After the ethanol had been driven off, the acid solution was made alkaline with ammonia and was extracted with chloroform. The residue after treatment with methanol gave the aglycone with mp 168-170°C (methanol), R_f 0.26, M^+ 427, identical with korsevine.

The alkaline solution after the isolation of the amino alcohol was neutralized with 5% sulfuric acid and evaporated to dryness. The dry residue was treated with ethanol. D-Glucose was detected in the ethanolic residue by descending paper chromatography in the butan-1-ol—pyridine—water (6:4:3) system [8] (Leningradskaya medlennaya ["Leningrad slow" (paper)]). The time of chromatography was 17 h. Aniline hydrogen phthalate was used as the revealing agent.

Acetylation of the Aglycone. Acetylation was carried out using 0.04 g of the aglycone, 1 ml of pyridine, and 1 ml of acetic anhydride, as for the acetylation of korsemine. This gave the amorphous acetyl derivative of the aglycone, identical with acetylkorsevine.

Mass spectrum: *m/e* 98, 111, 112, 113, 119, 121, 124, 139, 148, 149, 161, 162, 164, 180, 220, 236, 237, 276, 292, 293, 314, 316, 349, 350, 364, 409, 427, 439, $(M-17)^+$, $(M-15)^+$, $(M-1)^+$, M^+ 469.

SUMMARY

1. From the combined alkaloids of Korolkowia sewerzowii Regel we have isolated korsevine and new alkaloids, one (I) having mp 255-257°C and another which we have called korsemine (II).

2. On the basis of the results of a study of IR, NMR, and mass spectra of the alkaloids it has been established that compound (I) is severine N-oxide, and (II) has the structure of korsevine 3β-D-glucopyranoside.

LITERATURE CITED

1. K. Samikov, R. Shakirov, and S. Yu. Yunusov, Khim. Prir. Soedin., 367 (1976).
2. K. Samikov, R. Shakirov, and D. U. Abdullaev, Khim. Prir. Soedin., 269 (1976).
3. R. N. Nuriddinov and S. Yu. Yunusov, Khim. Prir. Soedin., 398 (1967).
4. K. Nakanishi, Infrared Absorption Spectroscopy: Practical, Holden-Day, San Francisco (1962).
5. R. N. Nuriddinov and S. Yu. Yunusov, Khim. Prir. Soedin., 258 (1968).
6. R. N. Nuriddinov and S. Yu. Yunusov, Khim. Prir. Soedin., 601 (1969).
7. D. U. Abdullaeva, K. Samikov, R. Shakirov, and S. Yu. Yunusov, Khim. Prir. Soedin., 671 (1977).
8. K. Samikov, R. Shakirov, K. A. Ubaidullaev, and S. Yu. Yunusov, Khim. Prir. Soedin., 183 (1975).

9. T. T. Gorovits, Khim. Prir. Soedin., 263 (1970).
10. W. Klyne, Biochem. J., 47, No. 4, xli (1950).

ALKALOIDS OF THE EPIGEAL PART OF Aconitum karakolicum

M. N. Sultankhodzhaev, L. V. Beshitaishvili,
M. S. Yunusov, and S. Yu. Yunusov

UDC 547.944/945

The dynamics of the accumulation of alkaloids in the epigeal part of Aconitum karakolicum according to vegetation periods has been studied. It has been established that the highest content of alkaloids is found in the early period. On separating the combined alkaloids, the aporphine alkaloid isoboldine has been isolated from this plant for the first time and a new base has been obtained — napelline N-oxide, the structure of which has been shown on the basis of the results of a study of spectral characteristics and direct transition to napelline.

Results have been given previously of a study of the alkaloids of the epigeal part of Aconitum karakolicum [1]. Attention was then directed to the fact that in the epigeal part of the plant the highly toxic aconitine is not the main component of the mixture of alkaloids, but the dominating alkaloids are those of the songorine group: napelline, acetylnapelline, and songorine itself [1, 2]. In view of the fact that the alkaloids of this group are of definite interest from the point of view of their physiological activity, we have studied the alkaloids of the epigeal part of Aconitum karakolicum collected in the upper reaches of R. Tyup (Kirghiz SSR) in various vegetation periods. Below we give information on the quantitative content of the total alkaloids (in percentages of the weight of the dry plant):

Vegetation Period and Date of Collection (1977)	Total Alkaloids, %
Early period, May 12	1.14
Budding, July 10	0.26
Flowering, August 5	0.22
Ripening of the seeds, August 25	0.17

The separation of the total alkaloids showed that in the early period the plant contains aconitine, aconifine, acetylnapelline, songorine, napelline, a base with mp 122-124°C having the composition $C_{19}H_{21}NO_4$, and a base with mp 197-199°C having the composition $C_{22}H_{23}NO_4$. On the basis of the results of a study of spectral characteristics, and also by direct comparison with an authentic sample, the base with mp 122-124°C was identified as the aporphine alkaloid isoboldine [3-5].

The alkaloid with mp 197-199°C was readily soluble in water and methanol, moderately soluble in chloroform and acetone, and sparingly soluble in ether. The NMR spectrum of the base showed the following signals: a three-proton singlet at 0.80 ppm ($>C{-}CH_3$), a three-proton triplet at 1.32 ppm ($N{-}C_2H_5$), and poorly resolved one-proton doublets at 5.09 and 5.25 ppm (terminal methylene group). The spectral characteristics of the alkaloid are close to those for napelline. The molecular weight of the base was 16 m.u. greater than that of napelline. This difference, and also the good solubility of the alkaloid in water and the presence in its mass spectrum of a characteristic triplet of peaks $M^+ - 16$, $M - 17$, and $M - 18$ permitted the assumption that the base was napelline N-oxide (I). In actual fact, when compound (I) was reduced with ferrous sulfate a product was obtained which was identified as napelline.

On separating the total alkaloids obtained in the budding period, we isolated the same alkaloids as in the earlier period, with the exception of aconifine.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 826-829, November-December, 1979. Original article submitted August 20, 1979.